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# Familial Amyloid With a Transthyretin Leucine 33 Mutation Presenting With Ascites

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**A 65-year-old female presented with symptomatic ascites. Light and electron microscopy examination of omental and peritoneal tissue obtained at exploratory laparotomy revealed amyloidosis. Immunochemical studies of the amyloid tissue showed positive staining with antibodies to transthyretin. Polymerase chain reaction (PCR), single strand conformation polymorphism analysis, and direct DNA sequencing demonstrated a transthyretin phenylalanine to leucine substitution at codon 33. This is only the second reported case of a transthyretin leucine 33 mutation. Moreover, this patient is unique among cases of transthyretin-associated amyloidosis with the clinical presentation of ascites. Am. J. Hematol. 59:249–251, 1998.** 

**Key words: amyloidosis; transthyretin; ascites**

## INTRODUCTION

Amyloidosis is a heterogeneous group of clinical and biochemical diseases characterized by the extracellular deposition of insoluble protein fibrils in tissues and organs [1–5]. Amyloidosis is characterized by a homogeneous, amorphous, eosinophilic substance under light microscopy, apple-green birefringence after Congo Red staining in a polarized microscope and aggregates of rigid, linear, nonbranching fibrils of 7.5–10 nm width and indefinite length in electron microscopy. Amyloid fibrils are derived from a variety of diverse and unrelated precursor plasma proteins. The classification of the different clinical forms of amyloidosis is based on the structural analysis of the fibrillar proteins and/or the gene coding for the precursor protein.

Familial transthyretin-associated amyloid (TTR-amyloidosis) is one of a group of hereditary forms of amyloid that is usually transmitted in an autosomal dominant manner [1,4–6]. Normal transthyretin (TTR) is a tetrameric protein composed of four identical subunits. Variant TTRs are usually the result of a single nucleotide based change (point mutation) in the TTR gene located at chromosome 18, leading to a substitution of a single amino acid in the mutant TTR molecule [1,4,6]. More than 50 TTR mutations are known to cause amyloid [1,2,4,7]. The TTR substitution of leucine for phenylalanine at position 33 (TTR leucine 33 mutation) has been described in only one previous case, reported by two groups [8,9].

Clinical manifestations of TTR-amyloidosis typically include a peripheral sensorimotor neuropathy usually beginning in the lower extremities, an autonomic neuropathy, and often cardiac involvement [1,5,6]. The present report describes a patient with TTR-amyloidosis caused by a TTR leucine 33 mutation who presented with symptomatic ascites and an elevated CA-125 level mimicking ovarian cancer. Extensive omental and peritoneal amyloidosis was discovered at laparotomy. This case is unique because to our knowledge no previous patient with TTR-amyloidosis has been reported with ascites from extensive peritoneal amyloid infiltration.

## CASE REPORT

A 65-year-old Polish-American female presented with a one-month history of dyspnea on exertion, abdominal swelling, and peripheral edema. The patient's family his-

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tory included a mother and father dying of ''old age'' in their 70s and four siblings who died of the following: hepatic cirrhosis at the age of 74, cancer of the colon at the age of 67, myocardial infarction at the age of 70, and stroke at the age of 74. A 40-year-old son is alive and well. Physical examination was significant for dullness to percussion and absent breath sounds at both lung bases, gross abdominal distention with ascites, and 3–4+ edema.

Abnormal laboratory studies included lactate dehydrogenase, 3.33 μkat/L (normal, 0.82–2.66), albumin, 29 g/L (normal, 40–60), and CA-125, 440 μ/ml (normal, <65). The serum total protein was 61 g/L (normal, 60–80). Immunoelectropheresis of serum and urine showed no monoclonal protein. A diagnostic paracentesis yielded clear yellow transudative fluid with a specific gravity of 1.009, protein, five g/L, and LDH, 0.21 μkat/L. No tumor cells were seen in the ascitic fluid. A chest roentenogram revealed bilateral pleural effusions. A computerized tomographic scan of the abdomen and pelvis showed a large amount of ascites. An electrocardiogram showed low voltage in the precordial leads.

A presumptive diagnosis of ovarian cancer was made. The patient underwent an exploratory laparotomy with total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and pelvic peritoneal biopsies. Histological examination revealed amyloid in all specimens. Electron microscopy studies of the omental tissue confirmed the diagnosis. No microscopic evidence of a malignancy was found. The cell block from the ascitic fluid showed reactive mesothelial cells that stained positive for CA-125 and negative for mucin, CEA, and CD-15.

Once the diagnosis of amyloid was made, additional studies were performed. A detailed neurological examination revealed a mild sensory neuropathy of the lower extremities. Electromyographic and nerve conduction studies of the upper and lower extremities showed evidence of a mild, distal, sensorimotor polyneuropathy of the axonal type in all limbs and bilateral carpal tunnel syndromes. A bone marrow biopsy was normal. A two-dimensional echocardiogram showed normal left ventricular chamber dimensions, near akinesia of the intraventricular septum, left ventricular ejection fraction of 50%, moderate concentric left ventricular hypertrophy (intraventricular septal 15–16 mm), biatrial enlargement, and normal right ventricular size and function. Immunochemical studies performed on the omental tissue showed positive staining with antibodies to serum amyloid P and transthyretin and negative staining for antibodies to antiamyloid A, beta-2 microglobulin, and kappa and lambda immunoglobulin light chains. The patient's pleural effusions resolved after surgery and removal of the ascites. However, the patient died suddenly at home two months later. A postmortem examination was not permitted.

## MATERIALS AND METHODS

DNA isolation from peripheral blood, polymerase chain reaction (PCR) amplification of segments of the TTR gene containing the TTR exons, single-strand conformation polymorphism (SSCP) analysis, and direct DNA sequencing of exon 2 PCR products were performed as described [10], except that 33 P-labelled nucleotides were used in the SSCP and sequencing reactions. In addition to performing SSCP analysis on the full-length PCR products for each of the exons, PCR products were digested with various restriction enzymes prior to electrophoresis to increase the sensitivity of the SSCP analysis.

## RESULTS

SSCP analysis of exon 2 PCR products digested with *Hae*III revealed that a mutation was present between the *Hae*III site in codon 29 and the 3′ end of the PCR product (in intron 2). SSCP of the PCR products derived from the other exons was normal. Direct PCR sequencing of the exon 2 PCR product revealed two bands at position 1 of codon 33, one corresponding to the normal sequence and the other to a G-to-A transition, encoding the amino acid change Phe to Leu (TTR Leu 33). To confirm the mutation, the exon 2 PCR product was sequenced in the opposite direction, and the same heterozygous mutation was seen. The remainder of the exon 2 sequence was normal.

## DISCUSSION

The present case report describes a unique patient with TTR-amyloidosis due to a TTR leucine 33 mutation whose initial clinical presentation included massive symptomatic ascites and an elevated serum CA-125 mimicking ovarian cancer. Exploratory laparotomy demonstrated extensive amyloid infiltration of the peritoneum and omentum associated with transudative ascitic fluid and reactive mesothelial cells staining positive for CA-125. No patient with TTR-amyloidosis has been previously reported with symptomatic ascites.

Ascites may develop in approximately 10–20% of patients with primary systemic (AL) amyloidosis [11,12]. Ascites in patients with AL amyloid has been attributed to congestive heart failure, nephrotic syndrome, or portal hypertension from advanced hepatocellular disease. In contrast, the present patient showed normal liver and renal function tests and her echocardiogram showed only a mild cardiomyopathy. The ascites in this present patient appeared to be due to amyloid infiltration of the perito-

neum and omentum. The elevated serum CA-125 level was likely derived from reactive peritoneum mesothelial cells that can secrete CA-125 [13].

TTR-amyloidosis is an autosomal dominant, hereditary disease of late onset with clinical symptoms generally beginning in the third decade of life or later [1,4–7,14,15]. The initial clinical features of TTR-amyloidosis typically include a peripheral sensorimotor polyneuropathy and an autonomic neuropathy. Orthostatic hypotension and gastrointestinal motility disturbances are common and variable degrees of cardiac, renal, gastrointestinal and ocular involvement can be found. Cardiac abnormalities include heart failure due to restrictive cardiomyopathy, arrhythmias, and EKG findings of low voltage, left axis deviation, conduction defects, and q and r wave abnormalities. Echocardiography shows a restrictive cardiomyopathy with thickened intraventricular septum and ventricular walls. Renal disorders may include proteinuria, nephrotic syndrome, and renal failure. Ocular findings are cataracts and vitreous opacities. Miscellaneous abnormalities have been weight loss, muscle atrophy, hearing nerve deafness, and carpal tunnel syndrome. The present patient demonstrated asymptomatic lower extremity polyneuropathy and bilateral carpal tunnel syndrome documented by electromyography and nerve conduction studies, an early cardiomyopathy on echocardiography, and low voltage on EKG due either to the cardiomyopathy or the pleural effusions.

One previous case of TTR-amyloidosis due to a TTR leucine 33 mutation has been reported by two groups [8,9]. Similar to our case, this patient was also of Polish-American ethnic origin, had no family history of amyloidosis, and had a late onset of symptoms at age 53. This patient initially developed paresthesias, sensory loss, and areflexia of the lower extremities due to a sensorimotor polyneuropathy along with constipation, impotence, and orthostatic hypotension due to an autonomic neuropathy. This patient's disease showed progression to an upper- and lower-extremity sensorimotor polyneuropathy and an infiltrative cardiomyopathy. The currently reported patient is unique with the initial clinical presentation of symptomatic ascites and asymptomatic mild peripheral neuropathy, carpal tunnel syndrome, and mild cardiomyopathy. This case demonstrates that the clinical picture of TTR-amyloidosis may be more diverse than previously reported. Evidence of widespread, generalized amyloid deposition in many organs may be found with TTR-amyloidosis [16]. Additional unusual clinical presentations may be reported as TTR-amyloidosis becomes more recognized.

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